08-16-01 JC20 Rec'd PCT/PTO 15 AUG 2001

FORM PTO-1390 (REV 11/98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NO. 1292 WO/US

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known see 37 CFR 1.5) 09/913788

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/01553	24 February, 2000	24 February, 1999

TITLE OF INVENTION
COMBINATION OF INTERCALATING ORGANOMETALLIC COMPLEXES AND TUMOR SEEKING BIOMOLECULES FOR DNA CLEAVAGE AND RADIOTHERAPY

APPLICANT(S) FOR DO/EO/US
MALLINCKRODT INC.

Applicant herewith submits to the United States Designed/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a filing under 35 U.S.C 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☑ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☑ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).
7. ☑ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☑ have been transmitted by the International Bureau
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☑ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☑ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☑ A FIRST preliminary amendment
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☑ A change of power of attorney and/or address letter.
16. ☐ Other items or information.

Express Mail # EL524584614US

U.S. APPLICATION NO (if known 37 CFR 1.5)	INTERNATIONAL APPLICATION NO		ATTORNEY'S DOCKET NUMBER
09/913788	PCT/EP00/01553	531 Rec'd PCT 15 AUG 2001	1292 WO/US

17. ☑ The following Fees are submitted:	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....**$1000.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....**$860.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$710.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....**$690.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33 (1)-(4).....**$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	24 - 20 =	4	x $18.00	$72.00
Independent claims	8 - 3 =	5	x $78.00	$390.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $270.00	$270.00
TOTAL OF ABOVE CALCULATIONS =				$1592.00
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$0.00
SUBTOTAL =				$1592.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$0.00
TOTAL NATIONAL FEE =				$1592.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +				$40.00
TOTAL FEES ENCLOSED =				$1632.00
			Amount to be refunded:	$
			charged:	$

a. ☐ A check in the amount of $ to cover the above fees is enclosed.

b. ☑ Please charge my Deposit Account No. 13-1160 in the amount of $1178.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-1160. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to Assistant Commissioner for Patents, Washington, DC 20231 on or before the following date:

Date Paula E. Ruch

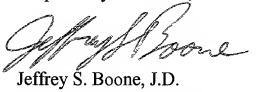
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

International Application No. PCT/EP00/01553
International Filing Date: 02/24/2000
Title: COMBINATION OF INTERCALATING ORGANOMETALLIC COMPLEXES AND TUMOR SEEKING BIOMOLECULES FOR DNA CLEAVAGE AND RADIOTHERAPY
Attorney Docket No.: 1292 WO/US

PRELIMINARY AMENDMENT

Please cancel any previous claims and replace with attached revised claims.

Respectfully submitted,



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August 6, 2001

What is claimed is:

1. The use of molecules that are taken up by the cell, which molecules comprise a tumor seeking biomolecule coupled to an intercalating moiety, which is complexed to a metal, which metal is preferably a radioactive metal, for the preparation of a therapeutic composition for the treatment and diagnosis of tumors and malignancies.

2. The use as claimed in claim 1 wherein the biomolecule is selected from the group consisting of somatostatin-, neurotensin-, bombesin-receptor binding molecules, antibodies, penetratines™, and molecules binding to the GPIIb/IIIa receptors.

3. The use as claimed in claims 1 or 2 wherein the intercalating agent is an aromatic molecule with an intercalative binding affinity for double-stranded DNA.

4. The use as claimed in claim 3, wherein the intercalating agent is selected from the group consisting of acridine, porphyrin, ellipticine, phenantroline, carbazole, benzimidazole or compounds with known cytostatic activity (antibiotics) from the class of tetracyclines (anthracyclines), such as daunorubicine, epirubicine or mixoxantrone.

5. The use as claimed in claims 1 or 2, wherein the radioactive metal is a γ-emitting nuclide.

6. The use as claimed in claim 3, wherein the radioactive metal is a γ-emitting nuclide.

7. The use as claimed in claim 4, wherein the radioactive metal is a γ-emitting nuclide.

8. The use as claimed in claim 5, wherein the radioactive metal is selected from the group consisting of Tc-99m, Re-186, Re-188 and Mn.

9. The use as claimed in claim 1, wherein the molecule has the general structural formula as given in Fig. 2.

10. The use as claimed in claim 1, wherein the molecule has any one of the structures as shown in Fig. 1.

11. The use of molecules as claimed in claim 1 for the preparation of a therapeutic or diagnostic agent for treating or diagnosing cancer tumors or malignancies.

12. A therapeutic composition, comprising one or more molecules as claimed in claim 1 and one or more suitable excipients.

13. A diagnostic composition, comprising one or more molecules as claimed in claim 1 in a suitable carrier.

14. A compound comprising

(a) a biomolecule molecule selected from somatostatin, neurotensin, bombesin-receptor binding molecules, antibodies, penetratines™, and molecules binding to GPIIb/IIIa receptors;

coupled to

(b) an aromatic intercalating moiety with binding affinity for double-stranded DNA selected from acridine, porphyrin, ellipticine, phenantroline, carbazole, benzimidazole, and tetracycline compounds with cytostatic activity;

which is complexed to

(c) a γ-emitting radioactive metal selected from Tc-99m, Re-186, Re-188, and Mn.

15. The use of the compound of claim 14 to diagnose a tumor.

16. The use of the compound of claim 14 to treat a tumor.

17. A kit for the preparation of a diagnostic or therapeutic composition comprising

(a) a biomolecule molecule selected from somatostatin, neurotensin, bombesin-receptor binding molecules, antibodies, penetratines™, and molecules binding to GPIIb/IIIa receptors;

coupled to

(b) an aromatic intercalating moiety with binding affinity for double-stranded DNA selected from acridine, porphyrin, ellipticine, phenantroline, carbazole, benzimidazole, and tetracycline compounds with cytostatic activity; and

(c) instructions to combine the above composition with a γ-emitting radioactive metal selected from Tc-99m, Re-186, Re-188, and Mn.

18. The kit of claim 17 additionally including one or more pharmaceutically acceptable excipients.

MOLECULES FOR THE TREATMENT AND DIAGNOSIS OF TUMOURS

The present invention relates to new molecules for the treatment and diagnosis of tumors. The invention furthermore relates to therapeutical compositions comprising one or more of these molecules and to the use of both in treatment and diagnosis of cancer.

The diagnosis and therapy of cancer still requires a large input from the pharmaceutical and chemical industry. Although a substantial effort is made to develop new treatments, there are still many tumor types for which no treatment exists. An additional problem is the formation of micrometastases, which cannot be diagnosed or treated.

An important problem in treatment is the similarity between normal cells and cancer cells. Treatments interfering with the growth of tumor cells will also interfere in the growth of healthy cells. Radiotherapy as it is now known consists essentially of an arbitrary cross-fire from outside the cell or the cytoplasm. Because this is a rather rough treatment surrounding cells and tissues might also be damaged leading to more or less severe side effects.

The provision of an improved radiotherapy and diagnostic method for cancer which uses very low amounts of radionuclides and leads to a direct treatment in the malignant cell is therefore highly desirable.

It is known that the metabolism of cancer cells differs from that of normal cells. In addition, cancer cells appear to have an increased membrane permeability in comparison to normal cells due to an increased expression of membrane receptors. The result is that the cancer cells are more permeable for biological vectors, like proteins and peptides.

The enhanced uptake of such biological vectors can be used in the diagnosis of tumors by binding a

radionuclide to a protein, for example by iodination of tyrosine functions in the protein or by covalent coupling of radioactive metal complexes. These molecules combine a tumor seeking function and a radioactive function. Although these types of molecules have been used for diagnosis, their use in therapy was not yet described.

It is the object of the present invention to further improve on the above described molecules to come to an even better tailored treatment of malignant cells.

This object is achieved by the invention by the provision of a molecule in which three functions are combined. This molecule comprises a tumor seeking molecule, which is coupled to an intercalating moiety, which is capable of complexing a metal, which metal is preferably a radioactive metal. The molecule can be targeted specifically to the tumor by the tumor seeking molecule and be internalized by the cell. The intercalating moiety will then insert into the DNA strand and induce breaks. In addition, the radioactive metal will also lead to strand breaking of the DNA. The advantage of the new molecules is that they are specifically directed to the malignant cell and are taken up by the cell.

The tumor seeking molecule is preferably a biomolecule, such as a peptide or protein that is actively targeted to the tumor cell. Examples of these biomolecules are somatostatin-, neurotensin-, bombesin-receptor binding molecules, monoclonal antibodies, penetratines™, and glycoproteins, and molecules binding to the GPIIb/IIIa receptors. The invention is however not limited to these examples and is more generally applicable to other tumor seeking agents as well. This category encompasses in addition compounds which are known to be transported into the nucleus or the nucleus membrane. Examples of these are anti-sense oligonucleotides, proliferating agents, like deoxy-uridine, and small molecules, like spermidine.

The intercalating moiety is preferably an aromatic molecule with an intercalative binding affinity

for double-stranded DNA. Examples of such aromatic compounds are compounds containing i.e. acridine, porphyrin, ellipticine, phenantroline, carbazole, benzimidazole or compounds with known cytostatic activity (antibiotics) from the class of tetracyclines (anthracyclines), such as daunorubicine, epirubicine or mixoxantrone and are functionalized with ligands able to coordinate the $[M(CO)_3]^+$ moiety. Examples of such ligands are those mentioned in EP-879 606 and additionally polyamino-polycarboxylates, phosphates and phosphonates, aliphatic or aromatic or mixed triamines and thiones.

The intercalating and tumor seeking functions are sometimes combined in existing molecules. Examples of intercalating agents combining an intercalating moiety and a peptide are actinomycin and triostin.

The radioactive molecule can be any radioisotope. Pure γ-emitting nuclides are preferred since their accompanying low range conversion electrons will lead to cleavage of bonds, which are close to the decaying nucleus. The dose burden to the patient remains thus very low.

Particularly suitable combinations of the three functions are given in Fig. 1.

The invention further relates to the use of the molecules in therapy and diagnosis and to therapeutical and diagnostic compositions comprising one or more of these molecules.

Therapeutical compositions comprise at least a suitable amount of the molecule in a diluent or excipient. Such compositions can take the form of solutions and are administered intravenously, intraperitoneally or intrathecally. Suitable amounts to be administered depend on the way of administration, the radionuclide used and the indication to be treated or diagnosed. Suitable amounts vary between 10^{-9} and 10^{-1} g per kg body weight.

Excipients and diluents for this type of medication are well known to the skilled person. However,

the present molecules require certain conditions for stability. Preferably, the excipient or diluent should be of a hydrophilic and preferably organic nature.

For diagnostic purposes the composition consists of at least a suitable amount of the molecule in a diluent or excipient. Diagnostic methods to be used with the composition of the invention are scintigraphy or Magnetic Resonance Imaging (MRI).

It was now found that the method for the synthesis of Tc and Re carbonyls from water described in EP-879 606 is suitable for preparation of the molecules of the invention. It is in particular possible with this method to introduce intercalating ligands, which form very stable complexes (in vitro and in vivo) with the above mentioned carbonyls. EP-879 606 is incorporated herein by reference.

The ligands claimed in EP-879 606 and acridine, porphyrin, ellipticine, phenantroline, carbazole, benzimidazole do stabilize the fac-$[Tc(CO)_3]^+$ moiety in serum and form complexes at very low concentrations. These ligands can be site specifically attached to the biomolecules and subsequently be labeled with i.e. Tc-99m. Since the radionuclide is very close to the intercalating ligand, its low energy electron will penetrate the DNA-strands very well and induce strandbreaking. When intercalating in one of the grooves, the probability to hit is very high since the nucleus is practically surrounded by DNA.

The biomolecules derivatized according to the invention exhibit high selectivity and are internalized. As known from pure organic intercalators, the complex is going to intercalate in DNA in particular when the cell is dividing. In contrast with other therapeutics, a high selectivity can be achieved with this combination.

If Re-188 is applied as the radionuclide, the damages will be much more severe than in the case of Tc-99m, but, consequently, the applied amount of radioactivity will be much lower than in case of "normal"

radiotherapy. Thus, severe side effects such as bone marrow toxicity could be avoided.

The present invention will be further illustrated in the examples that follow and which are solely intended to clarify the invention, but are in no way intended to be limiting to the scope thereof.

In the Examples reference is made to the following figures:

Figure 1: schematic representation of potential molecules of the invention.

Figure 2: example of a Tc(I) complex with this intercalating ligand and a potential biomolecule attached by direct linkage to another coordination site.

Figure 3: schematic representation of the method for preparing molecules of the invention.

Figure 4: schematic representation of the three types of plasmid structures.

Figure 5: ethidium bromide stained agarose gel of the three types I, II and III of DNA (left lane) and a molecular weight marker (right lane).

Figure 6: ethidium bromide stained agarose gel of a plasmid preparation treated with the compound $[^{99m}Tc(P_1)(teta)(CO)_3]$; The application site of the sample is on the bottom of the gel. Lane 1 is the molecular weight marker; lane 2 is a reference solution containing supercoiled, relaxed (single strand break) and linearized (double strand break) plasmid, lane 3 is the experimental solution containing both plasmid and the intercalator of the invention, and lane 4 is the negative reference containing only plasmid.

Figure 7: reaction scheme for the preparation of model bifunctional intercalators.

Figure 8: reaction scheme for the preparation of model trifunctional intercalators.

EXAMPLES

EXAMPLE 1

Synthesis of the molecules of the invention

1. Introduction

To provide a strong intercalation, the intercalator should be preferably planar and aromatic heterocyclic. Furthermore, pendant groups in the intercalator must stably be coordinated to the radionuclide (i.e. ^{99m}Tc). In this example, it is not coercive that the coordinating unit must be a multidentate ligand with high thermodynamic stability, since most complexes with Tc(I) show an extremely high kinetic stability. For these reasons and due to the already known principles of complexation of several mono- and bidentate ligands (especially picolinic acid) 5,6-benzochinolin-3-carboxylic acid was selected as intercalator.

Figure 2 depicts an example of a Tc(I) complex with this intercalating ligand and a potential biomolecule attached by direct linkage to another coordination site.

2. Synthesis of the example intercalator

2.1. **3-cyano-4-benzoyl-3,4-dihydrobenzo(f)chinoline 2**

648 µl (5.58 mmol) benzoyl chloride was added to a two phase system of water/methylene chloride over a period of two hours. These two layers contain 500 mg (2.79 mmol) of benzo(f)chinolin in the methylene chloride layer and 545 mg (8.37 mmol) KCN in water. Stirring was continued for 6 hours. The organic phase was separated and washed with water, 5% hydrochloric acid, water, 5% NaOH solution, and again with water. After drying over magnesium sulfate, the solution was evaporated to dryness.

The bromide salt of this so-called Reissert-compound was recrystallized from 95% ethanol to yield the analytically pure substance. Yield: 612 mg (71%).

2.2 5,6-benzochinolin-3-carbon acid (P1)

2 ml 48% hydrobromide acid were added to 287 mg (0.93 mmol) of the Reissert-compound dissolved in 2 ml acetic acid. The solution was refluxed during 24 hours, cooled and filtered. The filtered product was washed with diethyl ether, dried, and recrystallized from methanol to yield 169 mg (0.76 mmol) (82%) of the hydrobromide of the intercalator as a yellow solid.

2.3 Macroscopic synthesis of Technetium and Rhenium complexes with P1 (5,6-benzochinolin-3-carbon acid)

2.3.1 $[NEt_4][ReBr(P1)(CO)_3]$

A suspension of 102 mg (133 µmol) $[NEt_4][ReBr_3(CO)_3]$, 29.7 mg (133 µmol) P1 and 116 µl (226 mmol) of trioctylamine were refluxed in dichloromethane until a clear solution was achieved. After evaporation of the solution, the complex **5** was extracted into THF. After evaporization of THF the residue was washed with diethyl ether to remove trioctyl ammonium bromide. Yield: 63 mg (67%) of the yellow complex.

2.3.2 **$[Re(P_1)(H_2O)(CO)_3]$**

200.0 mg (0.26 µmol) of $[NEt_4]_2[ReBr_3(CO)_3]$ were refluxed in the presence of 29.1 mg of the intercalator P1 during 4 hours in 1M MES-buffer solution. Then the yellow precipitation was filtered. Yield: 114.2 mg (86%).

2.4 Microscopic synthesis of $[^{99m}Tc(H_2O)(P_1)(CO)_3]$

The ^{99m}Tc complexes were synthesized in a two-step procedure with a normal generator eluate. In a first step the complex was synthesized in >97% yield according to the literature (R. Alberto et al., J. Am. Chem. Soc. 120, 7987 (1998)). The solution was then neutralized with phosphate buffer in the reaction vial and a solution of the corresponding ligand was added. The end concentration was between 10^{-4} and 10^{-5}. It was left standing for 30 minutes at 75°C. The radio-chemical purification and the yield were defined through HPLC-

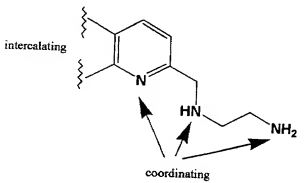
chromatography and it was discovered that $[^{99m}Tc(HPO_4)(P_1)(CO)_3]^{2-}$ (compound **10**) with a yield of 80-95% (dependent on the ligand concentration and the reaction time) was formed.

2.5 Synthesis of model trifunctional molecules of the invention

This is an example how a trifunctional molecule can be built. The procedure is based on known synthetic approaches for the corresponding coupling methods. The schematic procedure is given in Figure 3.

1. Syntheses of the **bifunctional** ligands, bearing an intercalator and a coordinating functionality

A bifunctional ligand was prepared according to the strategy described in Fig. 3. Fig. 7 gives the specific reaction scheme of the reaction that is described hereinbelow.



2-Methylquinoline (1)

2-Methylquinoline (**1**) was bought from Fluka and used without further purification.

Quinoline-2-carbaldehyde (2)

A mixture of 5.5 g of selenium dioxyde (49.5 mmol) in 50 ml dioxane and 2 ml water was added in small portions over 10 minutes to a boiling solution of 4.4 g (30.7 mmol) of 2-methylquinoline (**1**) in 20 ml dioxane. After 6 hours of boiling, the warm reaction mixture was filtered. The filtrate was evaporated, dissolved in

dichloromethane and filtered through Alox. The yellow-brown solid product obtained after evaporation of the solvent was recrystallized from dichlormethane. Yield: 3.76 g (78%)

^{1}H-NMR (DMSO):δ, 10.12s, 8.61d, 8.22d, 8.12d, 7.99d, 7.91t, 7.79t

Compound 3a

A mixture of 500 mg of quinoline-2-carbaldehyde (2) (3.2 mmol) and 330mg of N-(2-aminoethyl)-acetamid (3.23 mmol) in 15 ml of methanol was stirred for 2 hours at room temperature. The light brown solid product obtained was directly used for the next reaction. Yield: ~770 mg (~100%)

^{1}H-NMR ($CDCl_3$):δ, 8.57s, 8.21d, 8.13d, 8.10d, 7.85d, 7,75t, 7.59t

Compound 3b

A solution of 175 mg (4.62 mmol) of $NaBH_4$ in 10 ml of ethanol was slowly added over 2 hours to a stirred solution of 500 mg (2.07mmol) of **3a** in 30 ml ethanol at 0°C. This mixture was then stirred overnight at room temperature. The solid substance obtained after evaporation of the solvent was triturated with a 3M Na_2CO_3 solution. The desired light brown product (**3b**) was then extracted with dichloromethane. Yield: 382 mg (76%)

^{1}H-NMR ($CDCl_3$):δ, 8.15d, 8.05d, 7.81d, 7.71t, 7.54t, 7.35d, 6.84br, 4.21s, 3.50q, 3.02t, 2.02s

Compound 3c

A solution of 200 mg of **3b** (0.82 mmol) in 20 ml of 2N HCl was refluxed for 6 hours. The oil obtained after evaporation of the solvent was washed with ethanol to give the desired light brown solid hydrochloride salt **3c**. Yield: 203 mg (90%)

^{1}H-NMR (D_2O):δ, 8.40d, 7.95t, 7.76t, 7.59t, 7.49d, 4.57s, 3.46t, 3.34t

N-BOC-diethylentriamine (4)

A solution of 500mg (2.29 mmol) of di-tert-butyl dicarbonate ($(BOC)_2O$) in 30 ml dioxan was slowly added to a solution of 1.49 ml (1.42 g) (13.74 mmol) of diethylentriamine in 80 ml of dioxan at 10°C. The mixture was then stirred for 15 hours at room temperature. The desired product precipitated as an oil, which was then separated from the rest of the solution, dissolved in water, filtered, and extracted with dichloromethane to finally give the desired product as a light yellow oil. Yield: 260 mg (56%)

^{1}H-NMR ($CDCl_3$): δ, 5.15br, 3.25br, 3.18t, 2.77t, 2.69t, 2.63t, 1.76br, 1.41s, 1.19t

Compound 5a

A mixture of 140 mg of quinoline-2-carbaldehyde (**2**) (0.89 mmol) and 200mg of N-BOC-diethylentriamine (0.99 mmol) in 30 ml of methanol was stirred for 3 hours at room temperature. The solid obtained after evaporation of the solvent was then washed with water to obtain the desired light brown product. Yield: 304 mg (94%)

^{1}H-NMR (DMSO):δ, 8.32d, 7.97t, 7.73t, 7.71d, 7.57t, 6.65t, 4.33s, 3.08t, 2.97t, 2.85t, 1.28s, 1.09t

Compound 5b

A solution of 41 mg (1.08 mmol) of $NaBH_4$ in 10 ml of ethanol was slowly added over 2 hours to a stirred solution of 148 mg (0.43mmol) **5a** in 30 ml of ethanol at 0°C. This mixture was then stirred overnight at room temperature. The solid brown oil obtained after evaporation of the solvent was triturated with a 3M Na_2CO_3 solution. The desired light brown product (**3b**) was then extracted with dichloromethane. Yield: 136 mg (92%)

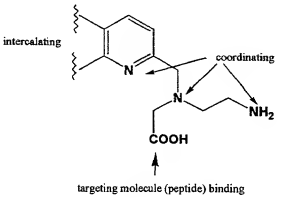
^{1}H-NMR (DMSO):δ, 8.29d, 7.94d, 7.92d, 7.71t, 7.61d, 7.54t, 6.71t, 3.95s, 2.96q, 2.59s, 1.33s, 1.22t

Compound 5c

A solution of 100 mg of **5b** (0.29 mmol) in 3N HCl was refluxed for 2 hours. The oil obtained after evaporation of the solvent was washed with diethylether to give the desired light brown solid hydrochloride salt **5c**. Yield: 102 mg (94%)
^{1}H-NMR (D_2O):δ, 8.44d, 7.95t, 7.77t, 7.6t, 7.51d, 4.51s, 3.44s, 3.34t, 3.27t

2. Synthesis of **trifunctional** model intercalators

Trifunctional intercalators were prepared starting from **5a** or **3b** of part 1 above. Fig. 8 gives the specific reaction scheme.



1. alkylation of an amine with bromo-aceticacid-ethylester

Amine **I** (Fig. 7; 547 mg, 2.83 mmol) and triethylene amine (0.510 ml, 3.08 mmol) were stirred in methanol (10 ml). The solution was cooled to 0°C, and ethyl bromoacetate **II** (0.313 ml, 2.83 mmol) was added dropwise within 5 minutes. After stirring the solution at room temperature for 18 hours, the solvent was removed in vacuo. The residue was dissolved in dichloromethane (50 ml) and washed three times with water (20 ml). The water phases were washed twice with dichloromethane (50 ml). The organic phases were dried over $MgSO_4$, filtered, and the solvent was removed in vacuo to give **III** as a yellow oil. Yield: 590 mg (2.11 mmol, 74.6%).
TLC (silica, ethanol) R_f 0.4

^{1}H NMR (200 MHz, d_6-acetone) δ = 8.44 (m, 1H, picolin), 7.65 (m, 1H, picolin), 7.45 (m, 1H, picolin), 7.21 (m, 1H, picolin), 4.12 (q, 2 H, J = 7.2 Hz, CH_2 ester), 3.94 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.32 (m, 2H, N-CH_2-CH_2-N), 2.82 (m, 2H, N-CH_2-CH_2-N), 1.84 (s, 3H, CH_3-CO), 1.21 (t, 3H, J = 7.2 Hz, CH_3 ester).

2. deprotection

Amine **III** (576 mg, 1.94 mmol) was dissolved ethanol (4 ml) and water (8 ml). NaOH 2M (2 ml) was added, and the solution was stirred at room temperature for 1.5 hours. Analytical HPLC exhibited a single peak, indicating that the ester group was cleaved quantitatively.

The solvent was removed in vacuo, the residue was dissolved in water (8 ml), and HCl 2N (1 ml) was added to neutralize the solution. HCl 33% (1.0 ml) was added, and the reaction mixture was stirred at 90°C for 48 hours. $NaHCO_3$ was added to neutralize the reaction mixture, the solvent was removed in vacuo and the residue was washed with ethanol. Removing of the solvent gave the deprotected product **V** as a yellow oil. Yield: 352 mmol (1.68 mmol, 68.6%).

^{1}H NMR (300 MHz, D_2O) δ = 8.44 (m, 1H, picolin), 7.85 (m, 1H, picolin), 7.45 (m, 1H, picolin), 7.39 (m, 1H, picolin), 3.78 (s, 2H, CH_2), 3.35 (m, 2H, N-CH_2-CH_2-N), 3.22 (s, 2H, CH_2), 3.32), 2.79 (m, 2H, N-CH_2-CH_2-N).

EXAMPLE 2

Strand breaking with the molecules of the invention in a model system

1. **Introduction**

1.1 The use of plasmids

To investigate the ability of the intercalating complexes with ^{99m}Tc to induce DNA-strandbreaks, plasmids were used as a model system. Plasmids are very suitable because electrophoretic analyses allow to differentiate

between double and single strand breaks. Additionally, large quantities of plasmids can be produced very simply by using cell biological methods.

A plasmid is a circular double-stranded DNA molecule, which double helical axis can be drilled into a superhelix. This form of the superhelix is described as type I. This type may loose its superhelix-structure by a single strandbreak and is then present as a relaxed circular DNA (type II). Through a double strandbreak of both types a linear from (type III) of the plasmid will be created. Figure 4 shows an example of the structure of these 3 DNA types.

Because these three DNA types have different structures, they may well be separated due to their size and especially their form by electrophoresis on agarose gel. The mixture (type I-III after the experiment) to be investigated is loaded on an agarose gel. A constant voltage will then be applied and the negatively charged DNA-fragments will migrate toward the cathode. The larger the form of the fragment, the slower the migration along the gel. DNA of type I (most compact) moves fastest, type II slowest. The gel will then be put in solution which contains very little ethidium bromide. The DNA fragments are made visible by intercalation and irradiation with UV-light of 300 nm depicting red-orange colored fluorescence (590 nm). This method is so sensitive that less than 5 ng DNA per band are detected. In the photographic record of the gels in Figure 5, the migration direction is from the top to the bottom.

1.2 Production of the plasmids

The plasmid Bluescript KS™ with a size of 2958 base pairs has been produced following the standard protocol of the company QIAGEN. Usually, this plasmid exists in the superhelix form (type I). With the restriction enzyme KpnI the linearized form of the plasmid DNA (type III) can be produced. A single strandbreak resulting in the relaxed circular form of

plasmid (type II), can be induced by the enzyme DNAase I. Figure 5, right lane, shows the electrophoresis on agarose-gel of a mixture of these three types of DNA. For the electrophoresis a marker with several sizes of DNA-pieces has been used as reference (left lane).

As demonstrated by the three bands of the right lane, the three types of DNA were clearly separated and can be distinguished after visualization. If single or double strand breaks result from conversion electrons, it should be easy detectable by this method.

2. Investigation of the ability of $[^{99m}Tc(P_1)(teta)(CO)_3]$ to induce strandbreaks

5 µl of a solution containing approx. 0.3 mCi/ml of $[^{99m}Tc(P_1)(teta)(CO)_3]$ and 100 ng of type I plasmid ($\sim=3^{*}10^{-5}$ M in base pairs) were left standing over a period of 18 hours. Then a electrophoresis of this mixture and the three references was made (Figure 6).

It is clearly visible that the plasmids in the measurement solution (lane 3) migrate slower than in the reference solution. The reason for this observation is that a small change in the structure of the plasmids is probably induced by intercalation of the complex into the double strand. This change in the tertiary structure of the plasmid did then allow a better intercalation of the ethidium bromide, thus explaining the stronger intensity of the band of sample solution.

Furthermore, in the comparison with the negative reference solution (lane 4), it is obvious that one or possibly two new bands appeared (arrows) in the lane of the solution treated with the ^{99m}Tc complex. The stronger of these two bands corresponds approximately to the position of type II on the band of reference solution in lane 2, containing the three types. This means that the complex has induced a single strand break in the plasmid.

Enclosure to letter dated 10 April 2001
International application No. PCT/EP00/01553

NEW CLAIMS

1. Use of molecules that are taken up by the cell, which molecules comprise a tumor seeking biomolecule coupled to an intercalating moiety, which is complexed to a metal, which metal is preferably a radioactive metal, for the preparation of a therapeutical composition for the treatment and diagnosis of tumors and malignancies.

2. Use as claimed in claim 1 wherein the biomolecule is selected from the group consisting of somatostatin-, neurotensin-, bombesin-receptor binding molecules, antibodies, penetratines™, and molecules binding to the GPIIb/IIIa receptors.

3. Use as claimed in claims 1 and 2 wherein the intercalating agent is an aromatic molecule with an intercalative binding affinity for double-stranded DNA.

4. Use as claimed in claim 3, wherein the intercalating agent is selected from the group consisting of acridine, porphyrin, ellipticine, phenantroline, carbazole, benzimidazole or compounds with known cytostatic activity (antibiotics) from the class of tetracyclines (anthracyclines), such as daunorubicine, epirubicine or mixoxantrone.

5. Use as claimed in claims 1-4, wherein the radioactive metal is a γ-emitting nuclide.

6. Use as claimed in claim 5, wherein the radioactive metal is selected from the group consisting of Tc-99m, Re-186, Re-188 and Mn.

7. Use as claimed in claims 1-6, wherein the molecule has the general structural formula as given in Fig. 2.

8. Use as claimed in claims 1-7, wherein the molecule has any one of the structures as shown in Fig. 1.

FIG. 1

Peptide
E
Tc
OC
CO
O
N
Intercalator
Peptide
H
N
Tc
OC
CO
Intercalator
backbone binder
NH
Tc
OC
CO
N
Anthracyclin
Peptide
NH
O
NH
Tc
OC
CO
N
Intercalator

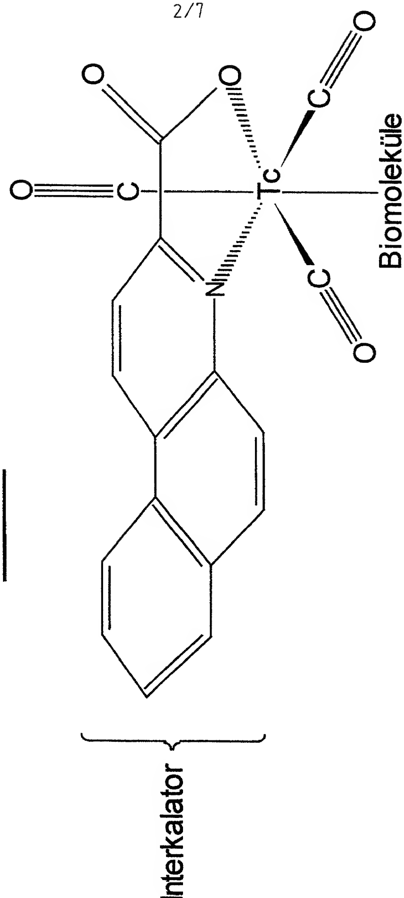


FIG. 2

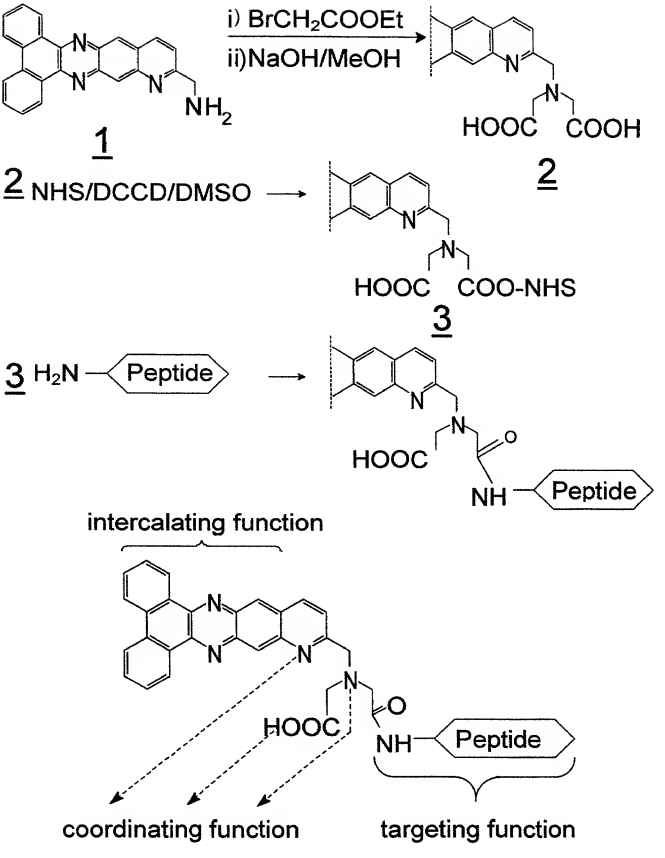

i) BrCH2COOEt
ii)NaOH/MeOH
NH2
1
HOOC COOH
2
2 NHS/DCCD/DMSO
HOOC COO-NHS
3
3 H2N Peptide
HOOC
NH Peptide
intercalating function
HOOC
NH Peptide
coordinating function
targeting function

FIG. 3

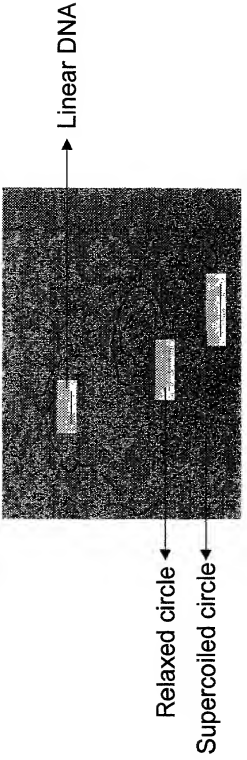


FIG. 4

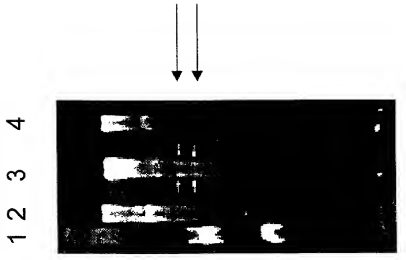


FIG. 6

FIG. 5

Reaction Scheme

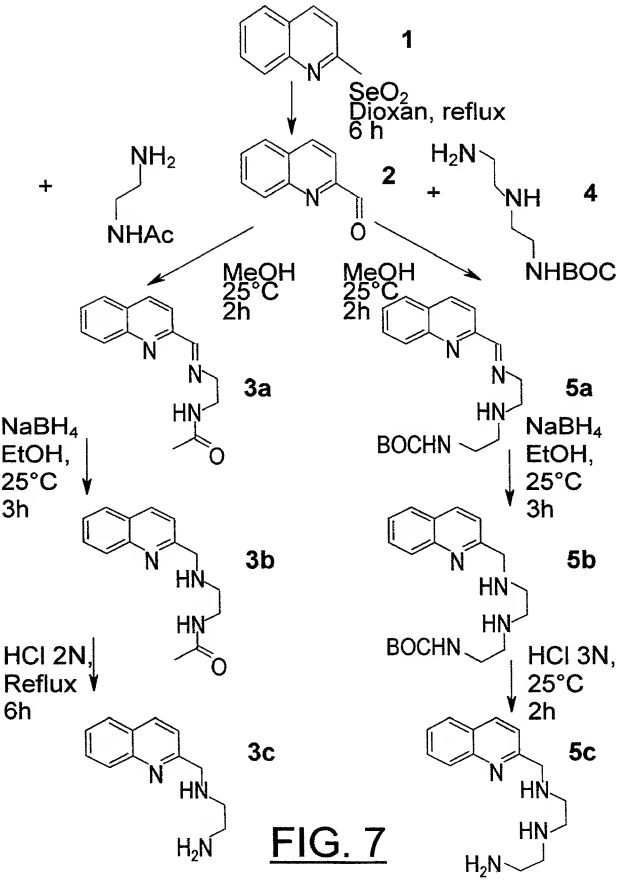


FIG. 7

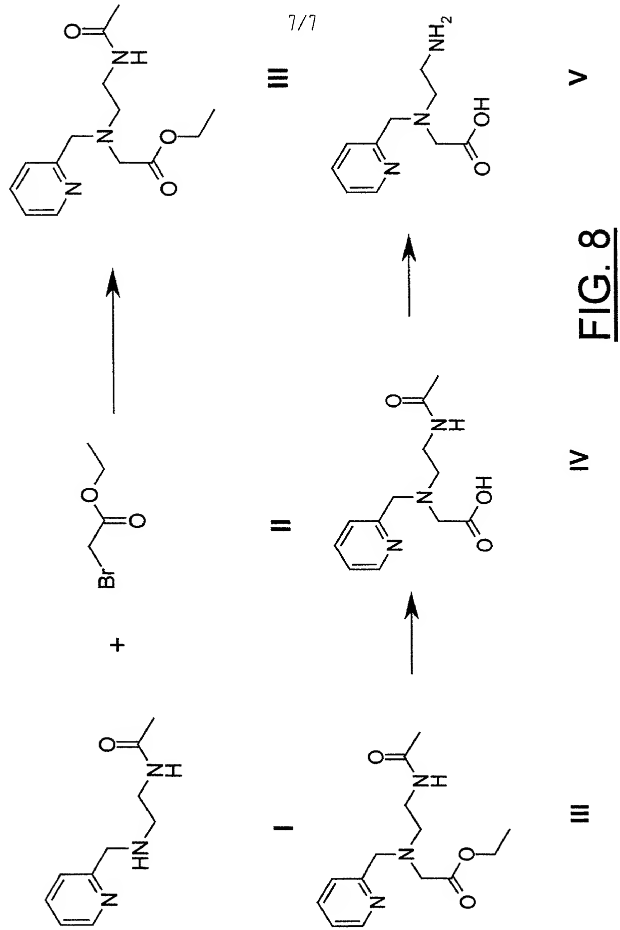


FIG. 8

PTO/SB/01 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

[X] Declaration Submitted with Initial Filing **OR** [ ] Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	1292 WO/US
First Named Inventor	ALBERTO, Roger
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

COMBINATION OF INTERCALATING ORGANOMETALLIC COMPLEXES AND TUMOR SEEKING BIOMOLECULES FOR DNA CLEAVAGE AND RADIO THERAPY

(Title of the Invention)

the specification of which

[ ] is attached hereto

OR

[X] was filed on (MM/DD/YYYY) 02/24/2000 as United States Application Number or PCT International Application Number EP00/01553 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
60/121340	USA Prov.	02/24/1999	[ ]	[ ]	[XXX]
99200754.2	EP	03/12/1999	[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]

[ ] Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

Patent Cooperation Treaty

Appointment of Agent

The undersigned applicant hereby appoints:

(2)

Lawrence L. Limpus Reg. No. 27,816

Jeffrey S. Boone Reg. No. 29,284

all of:

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675 McDonnell Boulevard
St. Louis, Missouri 63042
United States of America
Telephone Number: 314.654.3778
Facsimile Number: 314.654.3156

as agents to act on its behalf before the competent International Authorities in connection with any and all international applications filed by it and to receive payments on its behalf.

At: St. Louis, Missouri, United States of America

Date: 31 MARCH 1999

Mallinckrodt Inc.

Lawrence L. Limpus
Assistant Secretary

PTO/SB/01 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION — Utility or Design Patent Application

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] OR ☒ Correspondence address below

Name: Jeffrey S. Boone

Address: 675 McDonnell Boulevard, P.O. Box 5840

City: St. Louis | State: MO | ZIP: 63134

Country: USA | Telephone: 314-654-8955 | Fax: 314-654-3156

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]): Roger Ariel | Family Name or Surname: ALBERTO

Inventor's Signature: [signature] | Date: 20.7.01

Residence: City: Winterthur | State: | Country: Switzerland | Citizenship: Switzerland CH

Mailing Address: St. Georgenstrasse 54

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NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]): Pascal Jean-Marie | Family Name or Surname: HAFLIGER

Inventor's Signature: | Date:

Residence: City: Zurich | State: | Country: Switzerland | Citizenship: Switzerland

Mailing Address: Guggacherstrasse 42

City: Zurich | State: | ZIP: CH-8006 | Country: Switzerland

☐ Additional inventors are being named on the ____supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

PTO/SB/01 (03-01)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Attorney Docket Number	1292 WO/US
First Named Inventor	ALBERTO, Roger
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

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(Title of the Invention)

the specification of which

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99200754.2	EP	03/12/1999	[ ]	[ ]	[X]
			[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]

[ ] Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

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DECLARATION — Utility or Design Patent Application

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Given Name (first and middle [if any]): Roger Ariel | Family Name or Surname: ALBERTO

Inventor's Signature: | Date:

Residence: City: Winterthur | State: | Country: Switzerland | Citizenship: Switzerland

Mailing Address: St. Georgenstrasse 54

City: Winterthur | State: | ZIP: CH-8400 | Country: Switzerland

NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

2-60 Given Name (first and middle [if any]): Pascal Jean-Marie | Family Name or Surname: HAFLIGER

Inventor's Signature: P. Hafliger | Date: 30.07.01 CHX

Residence: City: Zurich | State: | Country: Switzerland | Citizenship: Switzerland

Mailing Address: Guggacherstrasse 42

City: Zurich | State: | ZIP: CH-8006 | Country: Switzerland

☐ Additional inventors are being named on the ____ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.